Aquaculture, 35 (1983) 125—135
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

MEASUREMENT AND POTENTIAL APPLICATIONS OF INDUCED TRIPLOIDY IN PACIFIC SALMON

FRED M. UTTER[1], ORLAY W. JOHNSON[1], GARY H. THORGAARD[2] and PETER S. RABINOVITCH[3]

[1] *Northwest and Alaska Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA 99164 (U.S.A.)*

[2] *Program in Genetics and Cell Biology, Washington State University, Pullman, WA 99164 (U.S.A.)*

[3] *Department of Pathology, School of Medicine, University of Washington, Seattle, WA 98195 (U.S.A.)*

(Accepted 12 December 1982)

ABSTRACT

Utter, F.M., Johnson, O.W., Thorgaard, G.H. and Rabinovitch, P.S., 1983. Measurement and potential applications of induced triploidy in Pacific salmon. *Aquaculture*, 35: 125—135.

Sterile triploids potentially could be useful in salmon culture because of the management options available with a sterile population and the ease with which triploidy can be induced. Heat shock applied for 10 min in a temperature range of 28—30°C and initiated 10 min following fertilization of eggs induced triploidy in coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*), and pink (*O. gorbuscha*) salmon, and in reciprocal hybrids between chinook and pink salmon as assessed by flow cytometric ploidy analyses. Moderate to high frequencies of triploid embryos were observed in all treated lots ranging from 58% (pink salmon) to 84% (one family lot of coho salmon). A substantial reduction in the frequency of triploid individuals between the eyed egg stage and 17 months was observed in one of two family lots of coho salmon. Significantly greater mean weight was observed at 17 months for diploid coho salmon (16.6 g) when compared to their triploid siblings (14.5 g). Mortality related to heat shock was observed primarily in coho salmon. Three triploid individuals were observed among 20 untreated pink salmon suggesting that triploid pink salmon may not be rare in natural populations.

INTRODUCTION

The idea of sterilizing part or all of the harvestable segment of a salmonid population is attractive to culturists and managers. A major benefit of such a capability is that metabolizable energy can be diverted from gamete production to somatic growth during the time when the population would normally mature, with a consequent improvement in flesh quality and reduction in

0044-8486/83/$03.00

mortality. Stocking of sterile fish also prevents the establishment of unwanted breeding populations or the disruption of genetic adaptions of indigenous gene pools through interbreeding with individuals that escape harvest.

Several methods have been used to sterilize salmonids. An effective but cumbersome method is surgical sterilization (Robertson, 1961; McBride et al., 1963; and Brown and Roberts, 1982). Although this method is limited by the requirement for individual surgery, these studies of surgical sterilization have clearly demonstrated the capability for extended survival and growth in Pacific salmon (*Oncorhynchus* spp.) in the absence of mature gonadal tissue.

Treatments involving pre-hatching immersion and post-hatching ingestion of appropriate levels of 17α-methyltestosterone have recently been shown to be effective for inducing sterility in Pacific salmon (Goetz et al., 1979; Donaldson and Hunter, 1982). Although such treatments appear to be highly promising means for sterilization, the requirement of somewhat prolonged exposure to androgenic hormones may limit their use in large-scale production of salmonids.

Other methods to induce the production of sterile triploids are also being attempted in large-scale sterilization of salmonids. One method is to create tetraploids through blockage of the first mitotic division of normally fertilized zygotes; mature tetraploids would then be crossed with diploid individuals to create triploid progeny. Tetraploid salmonid embryos have, in fact, been produced through mitotic inhibition by use of cytochalasin B (Refstie et al., 1977) and heat shock (Thorgaard et al., 1981). However, such treatments have resulted in large mortalities and generally abnormal development. Mature tetraploid rainbow trout (*Salmo gairdneri*) have been reported (Refstie, 1981), but the fertility of such individuals is uncertain. This procedure is further limited by the requirement for the retention of brood stock and a generation delay in the production of triploid progeny.

There are advantages to the direct production of triploid salmonids from diploid parents. Chemical treatments, as indicated above, have generally resulted in the production of tetraploids or polyploid mosaics (Refstie et al., 1977; Allen and Stanley, 1979). Cold shock treatments have been reported to produce either no viable polyploids (Refstie, 1981; Refstie et al., 1982) or polyploid mosaics (Lemoine and Smith, 1980).

The most successful means to date for direct induction of triploidy in salmonids have been through heat shocks. Chourrout (1980) reported that 50% of rainbow trout embryos treated at 27—30°C within the first hour after fertilization developed into triploids. Thorgaard et al. (1981) reported a similar proportion of triploid embryos in rainbow trout from heat shocks applied 10 min following fertilization. Subsequent modifications of these methods have produced lots of rainbow trout approaching 100% triploids (Chourrout and Quillet, 1982).

This paper describes the production of triploids through heat shocks in

three species of Pacific salmon and in the reciprocal hybrids of two of these species. The ploidy of large numbers of individuals is assessed by the use of flow cytometric DNA content analysis. We also measured the relative survival and growth of diploid and triploid salmon up to 18 months post-hatch and we discuss the potential applications of inter- and intraspecific triploids as management and research tools for Pacific salmon.

METHODS AND MATERIALS

Eggs and sperm of coho salmon (*O. kisutch*) were obtained at the Puyallup Hatchery, Washington State Department of Fisheries (WDF), in October 1980, and from fish returning to the Northwest and Alaska Fisheries Center in Seattle in November of 1980. On 19 October 1981, eggs and sperm were obtained from the WDF hatchery at Hoodsport, Washington, for pink salmon (*O. gorbuscha*) and from the WDF hatchery on the Cowlitz River for spring-run chinook salmon (*O. tshawytscha*). All gametes of individual fish were collected in separate containers and were stored on ice for periods of up to 6 h prior to fertilization. The crosses of coho salmon were made as full sibling families. Five individuals of each sex were collected for chinook and pink salmon crosses; eggs or sperm were pooled prior to fertilization because of logistical constraints, and crosses were made both within species and reciprocally between species.

Eggs were fertilized and water activated at 10°C. Volumes of sperm varied upward from a minimum of 1 ml/1000 eggs. Subsequent embryonic development in both heat shocked and control lots proceeded at ambient temperatures ranging from 6 to 12°C. Heat shocking was administered in a water bath at 10 min following water activation of the fertilized eggs under varying time and temperature regimes. Groups of crosses from the October collections of coho salmon were heat shocked for 1 min at 33, 34, or 35°C. Two families of the November collection of coho salmon were divided into subgroups. Different subgroups from each family were heat shocked for 10 min at 24, 26, 28, or 30°C. Crosses involving chinook and pink salmon were heat shocked for 10 min at 29°C (a presumably suitable temperature based on the earlier work with coho salmon).

Ploidy levels from eyed coho salmon embryos (summarized in Table I) were determined by chromosomal preparations. Methods followed the procedures outlined in Thorgaard et al. (1981).

All other ploidy analyses were carried out by flow cytometry which was performed in the Department of Pathology, University of Washington School of Medicine, and followed procedures outlined in Thorgaard et al. (1982).

Blood was drawn with a 22–25 gauge needle by cardiac or caudal artery puncture from yearling coho salmon into 0.5 ml of Alsever's solution. Only a small volume of blood (approximately 5 μl), sufficient to impart a faint coloration to 0.5 ml of a modified Alsever's solution (2% glucose, 0.8% trisodium citrate, 0.4% sodium chloride) was required for reliable

TABLE I

Ploidy levels and survival in heat shocked and untreated lots of coho salmon from two families

Family	Treatment (°C)	Age	Ploidy			Fertilization to eyed egg		
			2N (No.)	3N (No.)	% 3N	Total (No.)	Survival (No.)	Survival (%)
1	30		1	3	85	860	403	47
	28		1	8				
	26	Eyed	6	1	14			
	24		7	0	0	252	204	81
	Control		12	0	0	714	670	94
2	30		4	5	68	972	605	62
	28		2	8				
	26	Eyed	8	2	20	481	439	91
	24		7	0	0	557	500	90
	Control		12	0	0			
						Eyed egg—18 months		
1	30—28		17	4	19	403	79	20
	26	17 months	75	21	22	204	96	47
	Control—24		10	0	0	670	403	60
2	30—28		9	12	57	605	193	32
	26	17 months	22	3	12	439	102	23
	Control—24		15	0	0	500	236	47

determinations of ploidy levels; such a volume was readily obtained from the fry, by severing the tail and dipping the fish into the Alsever's solution.

A small volume of the blood cell suspension at the appropriate dilution was added to 1 ml of a solution of 10 g/ml diamidino-2-phenyl-indole (DAPI), a DNA specific fluorescent dye, in a buffer containing 0.146 M NaCl, 0.1 M Tris (pH 7.4), 1.0 mM $CaCl_2$, 0.21 mM $MgCl_2$, and 0.6% Noniodet P-40.

Suspensions of nonblood cells were obtained by vigorous mincing of small amounts of fresh tissue (e.g., 10 mg) in the DAPI–detergent solution. The suspended cells were passed twice through a 100 m steel mesh filter, syringed twice through a 26 gauge needle, and subsequently treated in the same manner as erythrocyte suspensions.

The DNA specific fluorescence of samples was quantified using a flow cytometer with UV excitation emission of 420 nm and above.

Ploidy levels were determined by comparing fluorescent peaks (based on individual determinations of thousands of cells) of known diploid individuals (drawn from untreated populations and previously tested by flow cytometry) with those of individuals of unknown ploidy levels.

RESULTS

A few general observations pertain to all experimental groups of this study. There were no obvious morphological distinctions between individuals

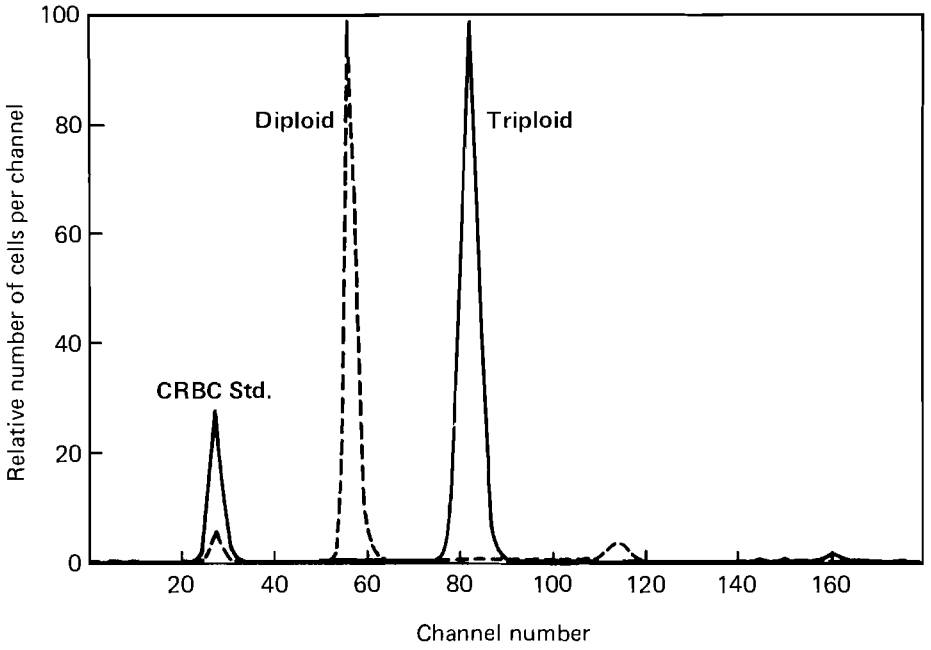

Fig. 1. Superimposed flow cytometric histograms of DNA fluorescence in erythrocyte suspensions from diploid (dashed line) and triploid (solid line) coho salmon and from chicken controls [CRBC STD (chicken red blood cell standards)]. Minor peaks reflect small populations of clumped erythrocytes of diploid (Channel 118) and triploid (Channel 160) cells frequently seen at 1—2% frequency in flow cytometric preparations.

in a particular treated group and those in its corresponding untreated group. None of the lots contained substantial numbers of obviously deformed individuals.

A test of ploidy levels determined by flow cytometry in different tissues of diploid and triploid individuals validated the general application of the method throughout this study. Identical expression of ploidy was observed in cellular preparations of liver, kidney, and adipose fins from two diploid and two triploid coho salmon where ploidy was initially determined by flow cytometry of erythrocyte suspensions. There was no evidence of mosaicism among these or any other cellular suspensions used in this study (Fig. 1).

Coho salmon

Our first attempt to induce triploidy in Pacific salmon (i.e., the October 1980 collection) was similar to procedures that had been successful in rainbow trout (Thorgaard et al., 1981). However, the 33, 34, and 35°C heat shocks for 1 min with the first group of coho salmon failed to produce any triploid progeny among 10 embryos that were randomly selected. Perhaps the larger size of the salmon eggs relative to those of rainbow trout

prevented induction of the effects of the heat shock during a 1-min exposure. These negative results led us to explore a longer exposure to a range of lower temperatures (Chourrout, 1980).

We applied this process to crosses made from the November collection and identified a temperature range (28—30° C) where a 10-min heat shock 10-min post-fertilization produced populations of eyed embryos where over half of the individuals sampled in each of the two families were triploid (Table I). The 28—30°C lots and the 24°C and control lots of each family were subsequently pooled because of space limitations.

A difference in the proportion of triploid individuals at the eyed egg stage and at 17 months was observed in the 28—30° C lots of the two families. A significant decrease in triploids occurred in family 1 (85% to 19%, $x^2 = 14$, 1 df, $P < 0.001$), while the proportions in family 2 remained similar (68% to 57%, $x^2 = 0.54$). No significant change occurred in the proportions of triploid individuals in the 26°C lots of both families through 17 months.

All individuals in the 26° C lot of family 1 that survived to 17 months were bled and weighed to compare the relative growth of diploid and triploid individuals reared in a common environment. The mean weight difference between the 75 diploids (16.6 g) and 21 triploids (14.5 g) was significant ($T = 2.527$, 53 df, $p = 0.0145$).

The mortality figures for the two families clearly indicated that the 28—30° C heat shock treatment increased the mortality in these lots during the early life history. This increase persisted from the eyed egg stage through 17 months.

Increased survival was seen in the 26° C lots of both families relative to that of 28—30° C groups. Survival approached or equalled that of the control groups during the early life stages, but mortality increased relative to the control group by 17 months in family 2.

Pink and chinook salmon

Data from the eight inter- and intraspecific combinations of heat shocked and unshocked lots involving eggs and sperm of pink and chinook salmon (Table II) indicated that triploids predominated over diploids in each of the heat shocked lots. A somewhat higher but nonsignificant proportion of triploids occurred in the two hybrid groups (83% and 70%) in comparison with the conspecific lots (60% and 58%).

None of the lots of untreated pure chinook salmon or hybrids contained triploid individuals; however, three of the 20 pink salmon from the untreated group were triploid. Spontaneous triploids have also been reported in rainbow trout (Cuellar and Uyeno, 1972; Grammeltvedt, 1974; Thorgaard and Gall, 1979).

Survival to 6 months in the treated groups of both the intraspecific and hybrid crosses relative to that in the control groups was substantially higher than was observed in similarly treated lots of coho salmon at the eyed egg

TABLE II

Ploidy levels and mortalities in heat shocked and unheated lots of chinook salmon (C), pink salmon (P), and reciprocal species hybrids at 5 months

Parent		Treatment	Ploidy			Survival to 5 months			x^2 (1 df)
M	F		2N (No.)	3N (No.)	% 3N	Total (No.)	Survival (No.)	Survival (%)	
C	C	Control	10	0	0	1155	1059	92	10.28**
		29°C	10	15	60	1690	1486	88	
P	P	Control	17	3	15	559	440	79	2.02
		29°C	6	15	72	289	215	74	
C	P	Control	20	0	0	81	60	74	5.60*
		29°C	5	28	85	149	129	87	
P	C	Control	22	0	0	1129	1031	91	241.03***
		29°C	7	16	70	1454	949	65	

*,**,***Indicate significance at 0.05, 0.01, and 0.001 levels, respectively, in survival comparison between control and test groups.

stage (Table II). Although both of the treated groups involving chinook salmon females had significantly lower survival than the control groups, the survival of the treated hybrid group involving pink salmon females was significantly higher than the control group. Only the treated hybrid group of chinook salmon females had mortality approaching that observed for the 28—30° C eyed egg lots of coho salmon.

DISCUSSION

Heat shocks have now been successfully used to induce triploidy in species of Pacific salmon used in this study as well as in rainbow trout (Chourrout, and Quillet, 1982; Lincoln and Scott, 1983). These common successes in inducing triploidy suggest that this procedure may be generally applicable to salmonids. The simplicity of induction of triploidy through short-term, moderate heat shock and the moderate to high proportions of resulting triploid individuals are appealing to potential users. Indeed, frequencies of triploids approaching 100% (79/80) have been induced in rainbow trout by methods similar to those that we used (Chourrout and Quillet, 1982). We will discuss below some of these potential applications as well as areas that require further investigation.

First, it is important to emphasize the major role that flow cytometry has played in the quantity, accuracy, and speed with which data were generated during this investigation. Chromosome counts accurately determine ploidy but are labor intensive and require the presence of actively dividing cells. Procedures measuring volume of erythrocytes (e.g., Lemoine and Smith, 1980) are faster than chromosome counts but the results are less certain because such measurements are only indirect reflections of actual

DNA content. However, each assay with flow cytometry accurately measured the DNA content of thousands of interphase cells in a few minutes and more than 50 determinations have been run in several hours; the directness and magnitude of such data thus give unerring determinations of ploidy levels equivalent to accurate chromosome counts. Reliable assays were made in this study from very small volumes of various tissues at developmental stages ranging from embryos to spawning adults. The use of flow cytometry is currently limited only by the availability of equipment; the procedure promises to become widely used for ploidy assays as the instrumentation becomes more available.

The absence of mosaicism in this study is consistent with observations of Thorgaard et al. (1982) involving the use of flow cytometry to study polyploid rainbow trout. These findings contrast with some other salmonid studies where mosaic individuals with both diploid and polyploid cells have been reported (Refstie et al., 1977; Allen and Stanley, 1979; Lemoine and Smith, 1980). It is possible that such mosaics are a reflection of different means used to induce polyploidy (i.e., cold shock; cytochalasin B treatment). Alternatively, the presumed mosaicism may be largely an artifact reflecting inherent limitations of the methods of measurement. Future reports of mosaicism should be verified by flow cytometry data.

The effects of heat shocking on other cellular processes require further study. Immediate effects are apparent in the 28—30°C lots of coho salmon while a delayed reaction appears to affect the 26°C lots. Comparable effects are less evident in the chinook and pink salmon at the same stages of development. The heat shock procedures are obviously effective in producing triploids, but additional experiments are required on a species by species basis through a range of time and temperature variables to optimize survival and triploid production.

The differential survival of diploids over triploids between the eyed egg stage and 17 months of rearing in the 28—30°C groups of coho salmon (family 1) is puzzling, considering the absence of such a difference over a similar period for the 26°C group of the same family. It is possible that other stresses from the higher levels of heat shock interacted more severely with the triploid condition resulting in differential mortality. The slower growth of family 1 triploids in the 26°C group is also consistent with such a possibility. A similar differential mortality and trend towards slower growth was observed in triploid rainbow trout by Thorgaard et al. (1982). Lincoln and Hardiman (1982) have also reported slower first year growth in triploid rainbow trout relative to controls. These negative effects of triploidy during early life history stages may be offset by the subsequent potential benefits of sterility during later stages [e.g., Thorgaard and Gall (1979) in rainbow trout; Wolters et al. (1982) in channel catfish *Ictalurus punctatus*].

Possible applications of sterile populations of salmon have been discussed in considerable detail elsewhere (e.g., Donaldson and Hunter, 1982). Both sterility and the potential for extended life and growth offer obvious advantages in numerous management situations.

Unknown ethological and ecological effects might also accompany production of triploids. What, if any, alterations would occur in migratory behavior? Could oversized and overaged individuals be adequately harvested to avoid excessive predation on existing resources including other salmonids? The economic and aesthetic appeal of an abundance of trophy salmon also requires considerations of such interactions. An orderly expansion following appropriate pilot studies is recommended rather than immediate massive releases of triploid individuals into presently stable marine environments. However, the sterility of triploid individuals precludes any permanent ecological disruptions from such releases.

Our primary rationale for making the hybrid crosses was to contrast the reciprocal effects of a single chromosomal dose of one species on two doses of another, with single doses of both species. The idea of blending the desirable qualities of two species is appealing to aquaculturists, and interspecific hybridization among Pacific salmon is well documented (see reviews of Dangel et al., 1973 and Chevassus, 1979). For instance, in the present crosses the pink salmon could add the capability for early seawater adaptation and the chinook salmon could superimpose more desirable flesh quality and large size. These crosses offer an additional dimension to previous studies of salmonid hybrids with the potential for increased viability in triploid hybrids through the presence of an additional maternal genome [as discussed in Elinson and Briedis (1968) and Bogart (1980), relative to amphibian hybrids, and inferred from the data of Campanna et al. (1974), where only triploids survived in crosses between brook trout, *Salvelinus fontinalis*, and rainbow trout]. The current data are inconclusive with regard to the possibility of increased hybrid viability in triploids. The survival data favor the treated group in hybrid lots involving pink salmon females, but favor the untreated group with the chinook salmon females. The frequency of triploids in the reciprocal hybrids was at least equivalent to that of the pure species, and growth and normal development relative to that of the diploid hybrid and pure species groups. Much of the evaluation of these crosses must await further growth and development.

We envision a promising future for induced triploidy in Pacific salmon as understanding of physiological mechanisms and ecological interactions increases. Advantages include triploid sterility; ease of triploid induction in a wide variety of salmonids; and potential for improved growth, longevity, and flesh quality in recreational and commercial fishes. These potentials remain to be tested for salmonids through production scale releases of fishes from public and private sector hatcheries.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contributions of the following individuals: Michael Shen, Department of Pathology, University of Washington, generously provided essential equipment, advice, and technical expertise for flow cytometry. Sue Paskell assisted in rearing the fish.

Gary Thorgaard acknowledges the support of the National Science Foundation Grant PCM 8108787 for his participation in this study.

REFERENCES

Allen, Jr., S.K. and Stanley, J.G., 1979. Polyploid mosaics induced by cytochalasin B in landlocked Atlantic salmon *Salmo salar*. Trans. Am. Fish. Soc., 108: 462—466.

Bogart, J.P., 1980. Evolutionary implications of polyploidy in amphibians and reptiles. In: W.H. Lewis (Editor), Polyploidy — Biological Relevance. Plenum Press, New York, NY, pp. 341—367.

Brown, L.A. and Roberts, R.J., 1982. Production of neutered salmonids. Comp. Biochem. Physiol. B, 73: 177—180.

Campanna, E., Cataudella, S. and Volpe, R., 1974. Un ibrido intergenerico tra trot iridea e salmerino di fonte (*Salmo gairneri* × *Salvelinus fontinalis*). Boll. Pesca, Piscic. Idrobiol., 29: 101—106.

Chevassus, B., 1979. Hybridization in salmonids: results and perspectives. Aquaculture, 17: 113—128.

Chourrout, D., 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri* Richardson). Reprod. Nutr. Dev., 20: 727—733.

Chourrout, D. and Quillet, E., 1982. Induced gynogenesis in rainbow trout: sex and survival of progenies production of all-triploid populations. Theor. Appl. Genet., 63: 201—205.

Cuellar, O. and Uyeno, T., 1972. Triploidy in rainbow trout. Cytogenetics, 11: 508—515.

Dangel, J.R., Macy, P.T. and Withler, F.C., 1973. Annotated bibliography of interspecific hybridization of fishes of the subfamily salmonidae. NOAA Tech. Memo. NMFS FNWS 1, 48 pp.

Donaldson, E.M. and Hunter, G.A., 1982. Sex control in fish with particular reference to salmonids. Can. J. Fish. Aquat. Sci., 39: 99—110.

Elinson, R.P. and Briedis, A., 1968. Triploidy permits survival of an inviable amphibian hybrid. Dev. Genet., 2: 357—367.

Goetz, F.W., Donaldson, E.M., Hunter, G.A. and Dye, H.M., 1979. Effects of estradiol-17β and 17α-methyltestosterone on gonadal differentiation in the coho salmon, (*Oncorhynchus kisutch*). Aquaculture, 17: 267—278.

Grammeltvedt, A.F., 1974. A method of obtaining chromosome preparations from rainbow trout (*Salmo gairdneri*) by leukocyte culture. Norw. J. Zool., 22: 129—134.

Lemoine, Jr., H.L. and Smith, L.T., 1980. Polyploidy induced in brook trout by cold shock. Trans. Am. Fish. Soc., 109: 626—631.

Lincoln, R.F. and Hardiman, P.A., 1982. The production and growth of female diploid and triploid rainbow trout. International Symposium on Genetics in Aquaculture, Abstracts, Univ. College Galway, Ireland, 29 March—2 April.

Lincoln, R.F. and Scott, A.P., 1983. Production of all-female triploid rainbow trout. Aquaculture, 30: 375—380.

McBride, J.R., Fagerlund, U.H., Smith, M. and Tomlinson, N., 1963. Resumption of feeding by and survival of adult sockeye salmon (*Oncorhynchus nerka*) following advanced gonad development. J. Fish. Res. Board Can., 20: 95—100.

Refstie, T., 1981. Tetraploid rainbow trout produced by cytochalasin B. Aquaculture, 25: 51—58.

Refstie, T., Vassvik, V. and Gjedrem, T., 1977. Induction of polyploid in salmonids by cytochalasin B. Aquaculture, 10: 65—74.

Refstie, T., Stoss, J. and Donaldson, E., 1982. Production of all female coho salmon (*Onchorhynchus kisutch*) by diploid gynogenesis using irradiated sperm and cold shock. Aquaculture, 29: 67—82.

Robertson, O.H., 1961. Prolongation of the life span of kokanee salmon (*Oncorhynchus nerka kennerlyi*) by castration before beginning of gonad development. Proc. Natl. Acad. Sci., 47: 609—621.

Thorgaard, G.H. and Gall, G.A.E., 1979. Adult triploids in a rainbow trout family. Genetics, 93: 961—973.

Thorgaard, G.H., Jazwin, M.E. and Steir, A.R., 1981. Polyploid induced by heat shock in the rainbow trout. Trans. Am. Fish. Soc., 110: 546—550.

Thorgaard, G.H., Rabinovitch, P.S., Shen, M.W., Gall, G.A.E., Propp, J. and Utter, F.M., 1982. Triploid rainbow trout identified by flow cytometry. Aquaculture, 29: 305—309.

Wolters, W.R., Libey, G.S. and Chrisman, C.L., 1982. Effect of triploidy on growth and gonad development of channel catfish. Trans. Am. Fish. Soc., 111: 102—105.